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Original Article

Circannual variability in adrenocorticotropic hormone responses to administration of thyrotropin-releasing hormone in clinically normal horses in Australia

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ABSTRACT

Thyrotropin releasing hormone (TRH)-stimulation testing for pituitary pars intermedia dysfunction (PPID) in horses is only recommended at certain times of the year. Current diagnostic cut-off values reflect testing in the northern hemisphere during this time. The aims of this study were to evaluate TRH stimulation testing during two different phases of the circannual pituitary cycle and to determine whether diagnostic cut-off values developed in the northern hemisphere are appropriate in Australia. Thirteen clinically normal horses at Perth, Western Australia, and 23 horses at Townsville, Queensland, Australia, had TRH stimulation tests performed at two different time points during the circannual pituitary cycle. At both locations, post-TRH adrenocorticotropic hormone (ACTH) concentrations were significantly different between testing time points (Perth: $P = 0.001$; Townsville: $P < 0.0001$). In Perth, the mean ACTH concentrations 10 min post-TRH in September and March were 51.4 pg/mL (95% confidence interval, CI, 46.4–56.4 pg/mL) and 248.5 pg/mL (95% CI 170.2–326.9 pg/mL), respectively. The median percentage change in ACTH concentrations in March was 361.9%. In Townsville, the mean ACTH concentrations 30 min post-TRH in September and April were 35.3 pg/mL (95% CI 29.6–40.9 pg/mL) and 112.3 pg/mL (95% CI 93.4–131.2 pg/mL), respectively. The median percentage change in ACTH concentrations in April was 144.7%. The ACTH cut-off value after TRH stimulation in normal horses in September in Perth and Townsville was similar to the values established in the northern hemisphere. However, TRH stimulation testing in March/April was highly variable at both locations.

Introduction

The circannual pituitary pars intermedia cycle affects baseline adrenocorticotropic hormone (ACTH) concentrations in both normal horses and horses with pituitary pars intermedia dysfunction (PPID) (Donaldson et al., 2005; Beech et al., 2009; Place et al., 2010; McFarlane et al., 2011; Copas and Durham, 2012; McGowan et al., 2013). More recently, this circannual cycle has been linked to changes in daylight length (Durham, 2014; Secombe et al., 2017), although other factors may also influence this rhythm. Broadly, between the summer and winter solstices, endogenous ACTH concentrations increase, reach a peak (acrophase) and then decline (Secombe et al., 2017). In contrast, between the winter and summer solstices, endogenous ACTH concentrations are lower and display reduced variability compared to the rest of the year (Durham et al., 2014a,b; Secombe et al., 2017). There are also increased ACTH responses to thyrotropin releasing hormone (TRH) administration between the summer and the winter solstices in clinically normal horses compared to the rest of the circannual pituitary cycle (Beech et al., 2007; Funk et al., 2011; Diez de Castro et al., 2014).

The ACTH response to TRH administration is a more sensitive test for PPID than baseline endogenous ACTH at certain times of the circannual pituitary pars intermedia cycle (Beech et al., 2007, 2011a,b). Several studies have shown that ACTH concentrations peak in less than 15 min post-TRH administration (Beech et al., 2007, 2011a,b). Current recommendations in the northern hemisphere between the winter and summer solstices support a

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Table 1
Means, 95% confidence intervals of the means and ranges of adrenocorticotropic hormone (ACTH) concentrations (pg/mL) for thyrotropin-releasing hormone (TRH) stimulation tests in September and March in Perth, Western Australia, prior to (T0) and 10 min after injection of TRH (T10).

	T0 September Perth	T10 September Perth	T0 March Perth	T10 March Perth
n	13	13	13	13
Mean ACTH (pg/mL)	26.7	51.4	49.6	248.5
95% Confidence interval	24.0–29.3	46.4–56.4	43.5–55.7	170.2–326.9
Minimum ACTH (pg/mL)	18.9	36.0	35.6	80.7
Maximum ACTH (pg/mL)	33.7	69.7	69.6	511.0

diagnostic cut-off value of 110 pg/mL ACTH at 10 min and 65 pg/mL ACTH at 30 min post-TRH administration.[1] The test may be used to differentiate normal horses from PPID-affected horses whose baseline endogenous ACTH concentrations fall within a 'grey-zone' (Durham et al., 2014b). In contrast to baseline endogenous ACTH testing, TRH stimulation testing is currently only recommended between the winter and summer solstices.

Although diagnostic cut-off values have been proposed for the northern hemisphere for the period between the summer and winter solstices (Haffner et al., 2014), a consensus has yet to be reached. Diagnostic cut-off values and seasonality of the TRH stimulation test have not been reported in the southern hemisphere. Recent data by the authors and others suggest that geographical location affects baseline endogenous ACTH upper reference limits (Copas and Durham, 2012; McGowan et al., 2013; Secombe et al., 2017). Whilst this may be due entirely to photoperiod differences, geographical or other climatic influences may also be involved. Previous research has identified differences in baseline pars intermedia secretions in different locations and climates (McFarlane et al., 2011), although their relative effects could not be separated.

The aim of the study was to characterise the endogenous plasma ACTH response to TRH stimulation at two time points of the circannual pituitary pars intermedia cycle in a group of clinically normal horses in two disparate geographical and climatic locations in Australia. The hypothesis was that performing the TRH stimulation test in close association with the spring equinox compared to the autumn equinox would result in a significant difference between the plasma ACTH concentrations at 10 min (T10) and 30 min (T30) post-TRH administration.

Materials and methods

Horses

The study protocol was approved by the animal ethics committees of Murdoch University (approval number R2702/14; date of approval 8th December 2014) and James Cook University (approval number A2127; date of approval 7th November 2014). Horses were selected at each of two geographical locations within Australia: (1) Perth in southern Australia (Western Australia; 31°57′S, 115°52′E; considered to be a hot-summer Mediterranean climate); and (2) Townsville in northern Australia (Queensland; 19°26′S, 146°81E; considered to be a tropical savanna climate).

In Perth, 13 horses were tested once in September 2015 and once in March 2016. Horses were chosen on the basis of age (<15 years), lack of clinical signs of PPID and normal baseline circannual endogenous ACTH concentrations. Previously, they had been used to determine the circannual ACTH reference interval at this location (Secombe et al., 2017). There were five Thoroughbreds (four geldings and one mare) and eight Standardbreds (four geldings and four mares). The mean age (range) was 10.4 (4–14) years. All horses were clinically well (based on monthly physical examinations) for at least 6 months prior to each time point. Horses were held on irrigated Kikuyu pasture in larger herds all year round with supplemental oaten hay. All horses were in moderate body condition at both testing times, with a median body condition score of 5/9 (range 4–6) (Henneke et al., 1983) when tested by two independent observers.

In Townsville, 23 horses were tested once in September 2015 and once in April 2016. Horses were similarly chosen on the basis of age, lack of clinical signs of PPID and normal circannual ACTH concentrations. All horses previously had been used to determine the circannual ACTH reference interval at this location (Secombe et al., 2017). Breeds represented were Thoroughbreds ($n = 14$; 13 mares, one stallion), Standardbreds ($n = 5$; all mares), Quarter Horses ($n = 3$; all mares) and an Australian Stock Horses ($n = 1$; mare). The mean (range) age of these horses was 11.4 (3–17) years. All horses were clinically well (based on monthly physical examinations) for at least 6 months prior to each time point. Horses were held under dry lot paddock conditions and received supplemental feed of roughage only or a balanced pelleted ration and roughage, depending on their level of physical activity. Horses were body conditioned during the April testing period by two independent observers; the median body condition score being 6/9 (range 4–8) (Henneke et al., 1983).

Collection and testing of specimens

Horses were normally held together in paddocks and were moved to smaller paddocks together on the morning of each test day at least 2 h prior to testing being performed. Evidence of excessive exercise or physiological stress was not observed in any of the horses. No supplemental feed (other than pasture) was supplied for the duration of the test. The horses were regularly housed as such as part of their teaching duties and were considered to be acclimatised to this procedure.

The TRH stimulation test was performed as described previously (Beech et al., 2007). Blood was collected into sterile plastic vacutainer tubes containing ethylenediamene tetraacetic acid (EDTA; Bio-One 4 mL plastic whole blood tube with spray-coated K_2EDTA, Greiner) for measurement of ACTH concentrations prior to intravenous injection of 1 mg of TRH (time 0, T0) (Sigma Pharmaceuticals). The TRH was reconstituted under a laminar flow hood with sterile water for injection to a final concentration of 1 mg of TRH/mL. The solution was filtered with a 0.22 μm syringe filter and stored in 1 mL aliquots at −80 °C until use (2–9 months). Blood was again collected into sterile plastic vacutainer tubes (Bio-One 4 mL plastic whole blood tube with spray-coated K_2EDTA, Greiner) containing EDTA at 10 min (T10; Perth) or 30 min (T30; Townsville) post-TRH injection and immediately placed on ice. The horses were monitored for 1–2 h and returned to their respective herds thereafter.

The samples were centrifuged in a refrigerated centrifuge for 5 min at 4000 *g* within 2 h of collection. The plasma was then separated and frozen at −80 °C until analysis. Plasma samples were transported on dry ice and ACTH concentrations were determined using a chemiluminescent assay (Immulite 1000, Siemens Healthcare) previously validated for use with equine plasma. Quality control materials provided by the manufacturer were used daily. The inter-assay coefficients of variation for lower and upper level quality control materials were 4.16% and 5.07%, respectively.

Statistical analysis

The data were assessed for normality using the Shapiro–Wilk test; all raw data were normally distributed either before or after logarithmic transformation. Normally distributed data are presented as means (±standard deviations; SDs) or means (ranges). The data for percentage change in ACTH concentration, evaluated between T0 and T10 or T30 at each time point, were not normally distributed and are presented as medians (ranges). A paired, two-tailed *t* test was performed to determine if a difference existed between the T10 or T30 ACTH concentrations at each two time point at each location. An unpaired two-tailed *t* test was performed to compare baseline ACTH concentrations between Perth and Townsville at both time points. A Wilcoxon signed-rank test was performed to determine if there were differences in the percentage increase in ACTH concentration after TRH stimulation between testing time points at each location. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using Medcalc Statistical Software version 16.4.3 and RStudio software.

Results

Four horses had one to two coughs post-TRH administration. No other adverse effects were noted. Results for Perth are presented in Table 1. In Perth, all baseline ACTH concentrations were within previously published reference intervals at both time points in the circannual pituitary pars intermedia cycle at this location

[1] See: https://sites.tufts.edu/equineendogroup/files/2015/12/2015-10-16_EEG-2015-recommendations.pdf (accessed 27 February 2017).